



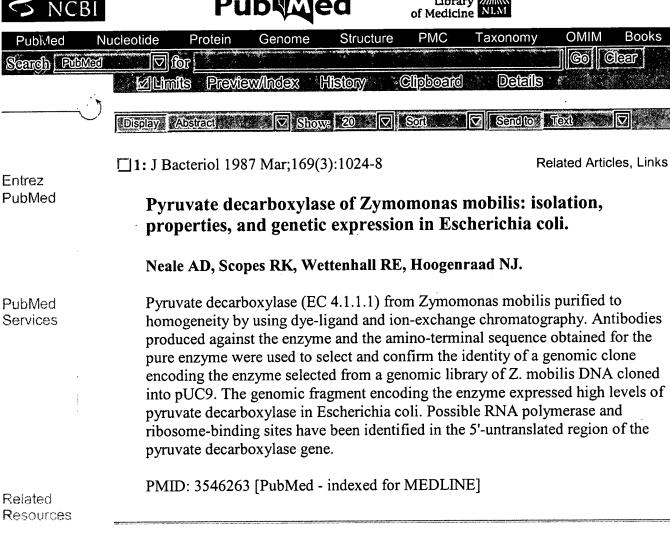


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		Structure and properties of pyruvate decarboxylase and site-directed mutagenesis of the Zymomonas mobilis enzyme.						
		Candy JM	I, Duggleby Ro	G.				
PubMed Centre for Protein Structure, Function and Engineering, Depar Services Biochemistry, University of Queensland, Brisbane 4072, Aust					t of			
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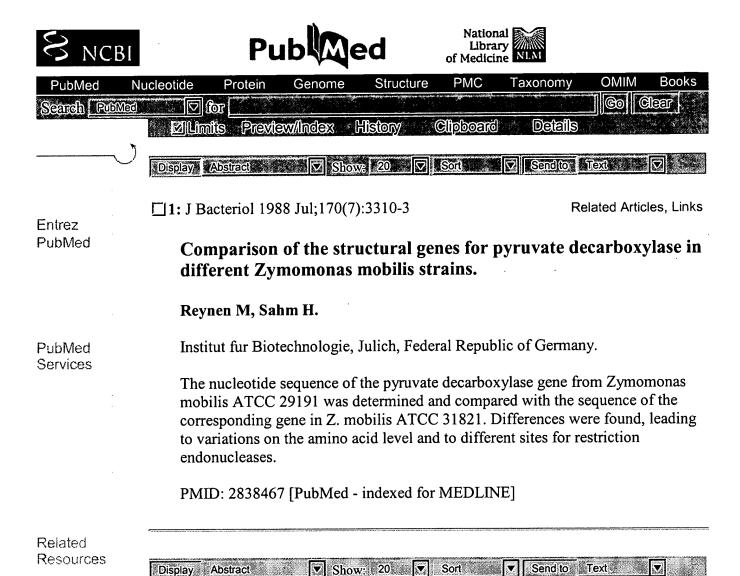
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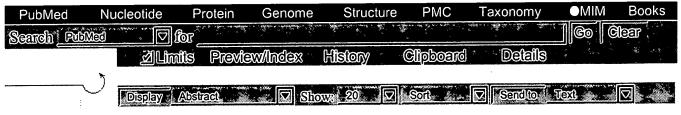
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	Neale AD, Scopes RK, Wettenhall RE, Hoogenraad NJ.						
PubMed Services	Pyruvate decarboxylase (EC 4.1.1.1), the penultimate enzyme in the alcofermentation pathway of Zymomonas mobilis, converts pyruvate to aceta and carbon dioxide. The complete nucleotide sequence of the structural gencoding pyruvate decarboxylase from Zymomonas mobilis has been det The coding region is 1704 nucleotides long and encodes a polypeptide of amino acids with a calculated subunit mass of 60,790 daltons. The amino sequence was confirmed by comparison with the amino acid sequence of selection of tryptic fragments of the enzyme. The amino acid composition obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with that obtained from the nucleotide sequence is in good agreement with the nucleotide sequence is in	oldehyde gene germined. 5567 o acid a					
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Cloning and sequencing of the gene coding for alcohol dehydrogenase of Bacillus stearothermophilus and rational shift of the optimum pH.

Sakoda H, Imanaka T.

PubMed Services

Department of Biotechnology, Faculty of Engineering, Osaka University, Japan.

Using Bacillus subtilis as a host and pTB524 as a vector plasmid, we cloned the thermostable alcohol dehydrogenase (ADH-T) gene (adhT) from Bacillus stearothermophilus NCA1503 and determined its nucleotide sequence. The deduced amino acid sequence (337 amino acids) was compared with the sequences of ADHs from four different origins. The amino acid residues responsible for the catalytic activity of horse liver ADH had been clarified on the basis of three-dimensional structure. Since those catalytic amino acid residues were fairly conserved in ADH-T and other ADHs, ADH-T was inferred to have basically the same proton release system as horse liver ADH. The putative proton release system of ADH-T was elucidated by introducing point mutations at the catalytic amino acid residues, Cys-38 (cysteine at position 38), Thr-40, and His-43, with site-directed mutagenesis. The mutant enzyme Thr-40-Ser (Thr-40 was replaced by serine) showed a little lower level of activity than wild-type ADH-T did. The result indicates that the OH group of serine instead of threonine can also be used for the catalytic activity. To change the pKa value of the putative system, His-43 was replaced by the more basic amino acid arginine. As a result, the optimum pH of the mutant enzyme His-43-Arg was shifted from 7.8 (wild-type enzyme) to 9.0. His-43-Arg exhibited a higher level of activity than wild-type enzyme at the optimum pH.

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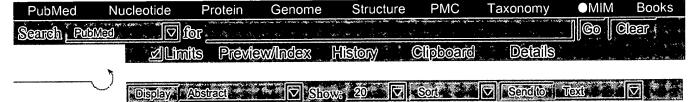
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☐1: J Biochem (Tokyo) 1996 Sep;120(3):498-504

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A thermophilic alcohol dehydrogenase from Bacillus acidocaldarius not reactive towards ketones.

D'Auria S, La Cara F, Nazzaro F, Vespa N, Rossi M.

PubMed Services Institute of Protein Biochemistry and Enzymalogy, C.N.R., Naples, Italy. e021rn02@area.ba.enr.it

An NAD-dependent alcohol-aldehyde oxidoreductase was purified to homogeneity and characterized from cell extracts of the thermophilic microorganism Bacillus acidocaldarius. The 500-fold purified homogeneous enzyme had a molecular mass of 154 kDa, as shown by gel filtration and glycerol gradient centrifugation. On sodium dodecyl sulfate polyacrylamide gel electrophoresis the protein showed one band of 38 kDa, indicating that the enzyme is a tetramer composed of subunits of identical molecular weight. Ethanol was the best substrate with the highest kcat/Km values, and the enzyme showed a substrate specificity that included linear, secondary and cyclic alcohols, as well as anisaldehyde, but it was not active on ketones. The protein contains eight zinc atoms per tetramer, four of which are removed by chelating agents with a concomitant loss of thermal stability. Circular dichroism spectra and determination of the NH2-terminal sequence allowed structural and homology comparison with other alcohol dehydrogenases from animal and bacterial sources.

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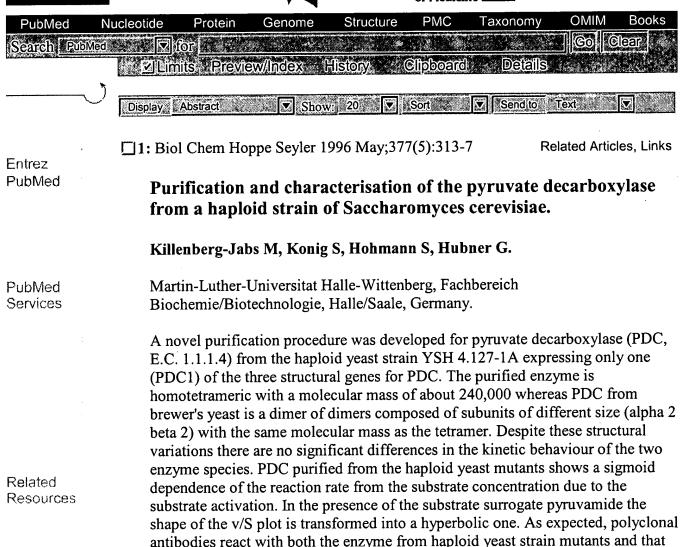
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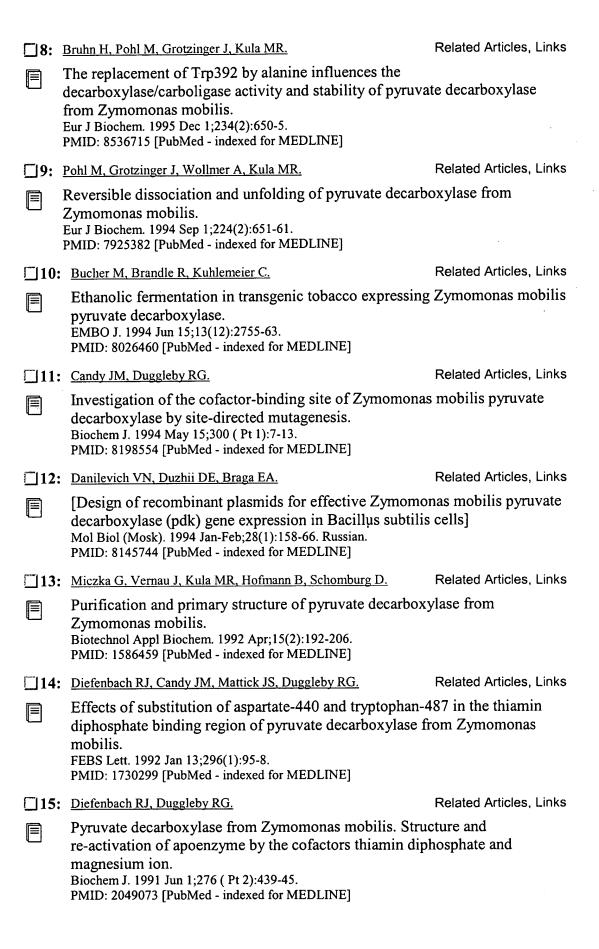
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:		Aspartate-27 and glutamate-473 are involved in mobilis pyruvate decarboxylase. Biochem J. 1999 Apr 15;339 (Pt 2):255-60. PMID: 10191255 [PubMed - indexed for MEDLINE]	n catalysis by Zymomonas				
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Services		Activation of thiamine diphosphate in pyruvate Zymomonas mobilis. FEBS Lett. 1998 Dec 28;441(3):404-6. PMID: 9891980 [PubMed - indexed for MEDLINE]	decarboxylase from				
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		Active site mutants of pyruvate decarboxylase is site-directed mutagenesis study of L112, I472, Eur J Biochem. 1998 Nov 1;257(3):538-46. PMID: 9839941 [PubMed - indexed for MEDLINE]					
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Resources	High resolution crystal structure of pyruvate decarboxylase from Zymobilis. Implications for substrate activation in pyruvate decarboxy J Biol Chem. 1998 Aug 7;273(32):20196-204. PMID: 9685367 [PubMed - indexed for MEDLINE]						
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		The role of His113 and His114 in pyruvate decomobilis. Eur J Biochem. 1997 Aug 15;248(1):63-71. PMID: 9310361 [PubMed - indexed for MEDLINE]					
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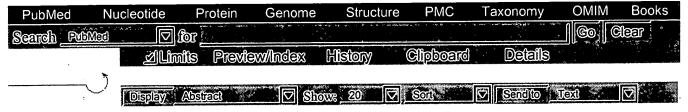
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1: Int J Biochem Cell Biol 1996 Feb;28(2):239-46

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RISEVIER SCIENCE FULL-TEXT ARTICLE

Purification and characterization of the alcohol dehydrogenase from a novel strain of Bacillus stearothermophilus growing at 70 degrees C.

PubMed Services Guagliardi A, Martino M, Iaccarino I, De Rosa M, Rossi M, Bartolucci S.

Dipartimento di Chimica Organica e Biologica, Universita di Napoli, Italy.

The biocatalysts isolated from thermophilic microorganisms are the object of ever-growing scientific interest for (i) the comprehension of the molecular basis of their thermal tolerance, and (ii) their use in different bio-industrial fields. Here we report the purification and characterization of an alcohol dehydrogenase (designated ADH-hT) from the novel strain LLD-R of Bacillus stearothermophilus which grows at 70 degrees C. ADH-hT was obtained in pure form by anion exchange chromatography and two affinity chromatographies, with a final yield of about 30%. ADH-hT was found to be a tetramer of 37 kDa-subunits, and to have a pI of 4.9. ADH-hT displayed a broad substrate specificity; its activity was highest for aldehydes, and decreased progressively for alcohols and ketones. ADH-hT was endowed with catalytic activity and resistance in the presence of several denaturing agents (organic solvents, detergents, chaotropic agents). ADH-hT shared with ADH 1503 (the alcohol dehydrogenase from B. stearothermophilus strain NCA 1503 which grows at 55 degrees C) the optimal temperature of 65 degrees C, but it was more resistant than ADH 1503 towards heating. In conclusion, due to its stability and broad substrate specificity ADH-hT could be utilized in bio-industrial processes. Furthermore, we believe that ADH-hT could represent a good model system for studying the mechanism(s) which proteins exploit to gain heat resistance.

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PubMed Services		DNA sequence analysis showed that pyruvate decarboxylase (one of the most abundant proteins in Zymomonas mobilis) contains 559 amino acids. The promoter for the gene encoding pyruvate decarboxylase was not recognized by Escherichia coli, although the cloned gene was expressed at relatively high levels under the control of alternative promoters. The promoter region did not contain sequences which could be identified as being homologous to the generalized promoter structure for E. coli. Hydropathy plots for the amino acid sequence indicated that pyruvate decarboxylase contains a large number of hydrophobic domains which may contribute to the thermal stability of this enzyme.						
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